CHARACTERIZATION OF FEED EFFICIENCY TRAITS AND RELATIONSHIPS WITH TEMPERAMENT, SERUM HORMONES AND SERUM METABOLITES IN GROWING BRANGUS HEIFERS

A Thesis

by

ROBYNNE RYAN GOMEZ

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2010

Major Subject: Animal Science

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Approved by:

Chair of Committee,	Gordon E. Carstens
Committee Members,	Luis O. Tedeschi
	Thomas H. Welsh, Jr.
Head of Department,	Gary R. Acuff

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ABSTRACT

Characterization of Feed Efficiency Traits and Relationships with Temperament, Serum Hormones and Serum Metabolites in Growing Brangus Heifers. (August 2010)

Robynne Ryan Gomez, B.S., Sam Houston State University

Chair of Advisory Committee: Dr. Gordon E. Carstens

Physiological traits that are biologically associated with feed efficiency may be useful indicator traits residual feed intake (RFI). The objective of this study was to examine the relationships between RFI, temperament, serum hormones and serum metabolites in growing heifers. A 4 yr study (n = 114-119 heifers/yr) was conducted with Brangus heifers (Initial BW = 271 ± 26 kg) that were weaned for 25.5 ± 8.6 d prior to high roughage diet adaptation (ME = 2.0 Mcal/kg DM). Individual dry matter intakes (DMI) were measured using Calan gate feeders and BW measured at 7-d intervals during the 70-d studies. RFI was calculated as the residual from the linear regression of DMI on mid-test $BW^{0.75}$ and average daily gain (ADG). Temperament scores and exit velocity (EV) were taken at 0-d. Temperament index (TI) was calculated as the average of EV and chute score. On 0-d, blood samples were collected and assayed for partial blood counts (WBC, RBC, hemoglobin, HB), metabolites (total protein, TP; glucose; creatinine; blood urea nitrogen, BUN; β-hydroxybutyrate, BHB) and hormones (cortisol; insulin-like growth hormone I, IGF-I). Across all heifers, RFI was positively correlated with DMI (0.70) and feed:gain (0.59). Heifers with low RFI (< 0.5 SD from mean RFI

0.00 ± 0.71 kg/d) consumed 16% less DMI and had 16% lower feed:gain than heifers with high RFI (> 0.50 SD from mean RFI). RFI was weakly correlated ($P < 0.05$) with WBC (0.15), HB (-0.11), total protein (-0.10), BUN (0.10), creatinine (-0.11) and BHB (0.13). Hemoglobin and BHB were weakly correlated with all feed efficiency traits except feed conversion ratio (FCR). No phenotypic correlation was found between cortisol and IGF-I with RFI. Temperament was not correlated with RFI. Cortisol, creatinine and glucose were moderately correlated with all temperament traits. Low TI heifers (calm) had significantly higher Final BW, ADG and DMI than high TI heifers. Calm animals had significantly lower cortisol, HB, creatinine and glucose and higher BHB. These results suggest that the temperament and serum metabolites evaluated in this study have limited utility as indicator traits for RFI in growing heifers.

DEDICATION

I wish to dedicate this thesis to my family for all their love and support through the years. To my Mama who is continuously present and always reminds me that I can accomplish anything with a little prayer and a glass of good wine. To my Daddy who always pushes me to excel in life but also taught me to enjoy the simple gifts around me. To my brothers, Roman and Roan, who keep me sane with their endless devotion and constant encouragement, yet manage to keep me humble in the process. To my sister-in-law, Rose, who is always compassionate, with a kind word and a positive manner. And finally to my nieces and nephews, Erin Ashley, Gabriel Roan, Megan Ryan, Rheana Katarin, and Ryan Gabriel who bring me endless joy and heartfelt pride in being your Tía. I would not be the person I am today without each of you and I thank God for bestowing you in my life. Thank you.

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NOMENCLATURE

ADG	Average daily gain
AST	Aspartate aminotransferase
BW	Body weight
BHB	Beta-hydroxybutyrate
BUN	Blood urea nitrogen
CP	Crude protein
CORT	Cortisol
CRT	Creatinine
CS	Chute score
DM	Dry matter
DMI	Dry matter intake
EV	Exit velocity
FBW	Final body weight
FCR	Feed conversion ratio (kg feed/kg gain)
GE	Gross energy
GGT	Gamma glutamyltransferase
GLUC	Glucose
HB	Hemoglobin
HCW	Hot carcass weight
IBW	Initial body weight

IGF-I	Insulin-like growth factor I
NDF	Neutral detergent fiber
MBW	Metabolic body weight
ME	Metabolizable energy
RBC	Red blood cell
REV	Relative exit velocity
RFIp	Residual feed intake for maintenance and growth
RFIs	Residual feed intake adjusted for serum parameters
TI	Temperament Index
TP	Total protein
WBC	White blood cell
WT	Weight

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CHAPTER I

INTRODUCTION AND

LITERATURE REVIEW

The profit margin for beef production is the difference between revenue received for weaned, stocker and (or) fed cattle and the costs of feeding and managing these cattle. The only means to increase these profit margins is to increase product output or decrease cost inputs. From 2003 to 2007, the yearly average price of corn increased from $2.59 to $4.15 a bushel, and due to droughts and loss of land for agriculture, the annual price of hay increased from $74 to $142 per ton. As feed costs continue to rise, there is a greater need to produce more efficient cattle that consume less feed and gain more muscle (lean product). Crews (2005) studied the economic benefits of feeding efficient vs. non-efficient cattle and found that it costs $38 more to feed a less efficient animal for 150 days than an efficient animal, which means that considerable costs could be reduced by shifting production to utilize more efficient animals. Moreover, research has shown that efficient cattle produce less manure and methane, and thus will have less of an environmental impact. Therefore, strategies that seek to improve the efficiency of feed utilization in beef cattle will benefit the beef industry in many ways.

Feed Efficiency in Growing Cattle

Efficiency in beef cattle production is defined as the ratio of outputs to inputs. Simply stated, outputs consist of body weight gain or lean product gain and inputs

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This thesis follows the style of Journal of Animal Science.

consist primarily of total diet consumed. The primary method of measuring feed efficiency utilized in the industry is feed conversion ratio (FCR), which is the ratio of feed intake to gain. Feed conversion ratio is moderately heritable (0.24 – 0.46; Bishop et al., 1991 and Arthur et al., 2001b) and is useful in assessing the effects of diet quality, environment and management practices on production efficiency in growing and finishing cattle (Carstens and Tedeschi, 2006). The use of FCR is extensive; however, there are disadvantages to its use as an efficiency trait for use in breeding programs. Feed conversion ratio has been shown to increase with age of the animal and be highly correlated with average daily gain (ADG) and body weight (Koots et al., 1994), such that selection for favorable FCR would lead to increased mature size and increased energy requirements for maintenance (Herd and Bishop, 2000). Mrode et al. (1990) conducted a study to look at this effect and concluded that selection based on feed consumed per unit or lean growth resulted in a correlated increase in cow size.

Residual Feed Intake

In response to this negative aspect of FCR, Koch et al. (1963) suggested an efficiency trait measure that takes into account variation in the composition of gain and maintenance requirements. Efficiency was expressed as gain adjusted for differences in feed consumption, +/- deviation from the regression of gain on consumption (Koch et al., 1963). Meaning selection for improved feed efficiency will reduce feed inputs without changing genetic potential for live weight and growth (Davis and Simmen, 2006). Residual feed intake (RFI) was originally defined as the difference obtained when actual feed intake is adjusted for growth and maintenance requirements. Through further

research, the definition of RFI has evolved into the difference between actual feed intake and expected feed intake calculated by linear regression of feed intake on growth rate and body size. To clarify, efficient animals are those that consume less feed than expected based on their size and growth rate, thus efficient animals will have a negative or low RFI. In contrast, inefficient animals will consume more feed than expected and have a positive or high RFI. Residual feed intake has been shown to be moderately heritable with heritability estimates ranging from 0.25 to 0.47 (Pitchford, 2004; Arthur et al., 1997; Arthur et al., 2001a, Lancaster et al., 2009). One of the major advantages of RFI is that it is phenotypically independent of the production traits used to calculate expected intake; this allows selection for a more efficient animals without affecting mature body weight, as seen in FCR. Cattle with low RFI have 6 – 18% lower FCR than cattle with RFI (Richardson et al., 1996; 2000; Richardson and Herd, 2004; Lancaster et al., 2009a). In addition, high genetic correlations exist between RFI in young cattle and RFI in the same adult animal where as feed conversion ratio is not correlated in the same animal at different ages or levels of production (Archer et al., 2002). Therefore, RFI may reflect more variation in metabolic processes rather than variation in levels of production.

Sources of Biological Variation in RFI

Herd and Arthur (2009) summarized the possible processes that could account for the variation in RFI: intake of feed, digestion of feed, metabolism, activity and thermoregulation (Figure 1). Richardson and Herd (2004) found that low RFI animals had higher digestibilities which they believe might allow higher amino acid availability

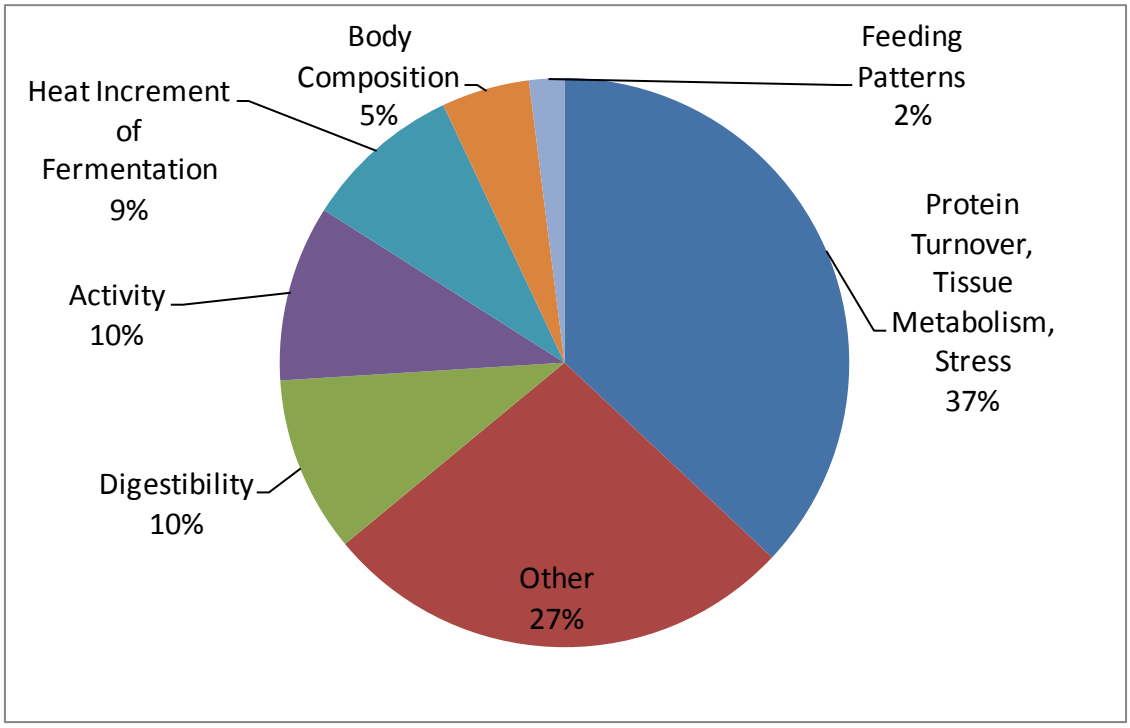

Figure 1.1 Contribution of various mechanisms to the variation in residual feed intake (Herd and Arthur, 2009).

to the microbial population in the rumen for protein metabolism. Another biological mechanism that accounts for variation in RFI is differences in composition of growth. Several studies have shown that cattle with low RFI have similar lean tissue growth rates but deposit less fat (Arthur et al., 2001a; Nkrumah et al., 2004). This variation in composition of gain (lean v. fat) and resulting body composition can influence the efficiency of nutrient utilization (Herd and Arthur, 2009). To further explain this variation between lean muscle growth and fat deposition, researchers have looked at metabolite concentrations in these divergent groups. They found that serum leptin concentrations, an adipose derived hormone, are positively correlated with RFI, and creatinine levels, a muscle mass marker, are highly negatively correlated with RFI (Richardson et al., 2004). As RFI decreases and animals become more efficient, adipose hormone levels decrease and muscle marker levels increase. Differences in activity level between animals with divergent RFI also accounts for differences in heat production levels. High RFI animals tend to be more active, ruminate longer and consume more meals per day which in turn causes a higher energy expenditure/heat production in these less efficient animals (Herd et al., 2004; Richardson et al., 2000). Similar observations were seen by Richardson et al. (2001) and Arthur et al. (2001b) in which they reported 10% of the observed variation in RFI was explained by daily pedometer count and was 5% explained by daily distance walked and time spent standing and ruminating. Given that the cost of measuring individual feed intake can be substantial, wide spread implementation of RFI as a feed efficiency trait has not been established. Knowledge of

the biological and physiological differences amongst RFI groups will provide further insight and allow early identification of the more efficient (low RFI) cattle.

Hematological Sources of Variation in RFI

Physiological indicators of economically relevant traits, such as ADG and feed intake, would be useful in predicating RFI and subsequent selection of efficient cattle as well as reduce the cost of measuring individual feed intakes. Researchers have examined blood cell profiles, serum hormones, and metabolite concentrations as possible physiological biomarkers. It has been suggested that insulin-like growth factor I (IGF-I), a polypeptide hormone, could be used as an indicator trait for RFI (Moore et al., 2005; Johnston et al., 2001). Concentrations of IGF-I regulate growth rate as well as glucose and amino acid metabolism during all stages of life. Elsasser et al. (1989) reported lower levels of circulating IGF-I in animals fed a restricted diet and lower levels of crude protein. Plasma IGF-I has been shown to be moderately to highly heritable (0.31-0.52) in beef cattle (Herd et al., 1995; Davis and Simmen, 2006), and associated with ADG, body size, carcass weight, feed conversion ratio and milk production (Davis et al., 1995; Johnston et al., 2001). Moore et al. (2005) reported a moderate positive genetic correlation ($r = 0.41$) with RFI and serum IGF-I concentrations in bulls and heifers. Similarly, Brown et al. (2004) reported positive phenotypic correlations with serum IGF-I concentrations and RFI ($r = 0.38$) and FCR ($r = 0.36$) in growing bulls. Wood et al. (2004) compared selection strategies using RFI and IGF-I in connection with other traits, and found that testing for IGF-I concentrations and RFI resulted in increased profits and suggested that testing for IGF-I allows producers to initially screen bulls so as to limit

the number of feed intake tests required. In contrast to these studies, Moore et al. (2005) reported that IGF-I concentrations were negatively correlated with growth traits, meaning that more efficient (low RFI) cattle had higher BW and lower levels of circulating IGF-I. Following a 5 year study with divergently selected cattle for IGF-I, Davis and Simmen (1997) found a negative correlation of post weaning IGF-I concentrations with final BW ($r = -0.31$) and ADG ($r = -0.40$) in Angus cattle. However, after 10 years of selection, they reported positive correlations between IGF-I concentrations and final BW and ADG ($r = 0.28$ and 0.29, respectively; Davis and Simmen, 2006). The researchers attribute the discrepancy in correlations to the change in selection lines over time.

Differences in metabolism are thought to account for a significant amount of variation in RFI. These metabolic factors are involved in protein metabolism, skeletal muscle growth, liver function, stress response, and energy utilization. Beta-hydroxybutyrate (BHB), which is often referred to as a ketone body, is produced during lipolysis and amino acid catabolism and can be used as an energy source in the absence of glucose in peripheral tissues. Acetoacetate can be reduced to beta-hydroxybutyrate by beta-hydroxybutyrate dehydrogenase in a NADH-requiring reaction. The extent of this reaction depends on the state of the NAD pool of the cell. Presence of BHB in the blood indicates that cells are glucose depleted and therefore require a subsequent energy source. Elevated levels of ketone bodies results in a condition, known as ketosis, which arises from starvation, or exercising to the limits of cellular carbohydrate depletion. Reist et al. (2002) studied the effects of energy balance on milk and blood traits in high-

yielding dairy cattle, and found a negative correlation with BHB levels, so as energy balance decreased, the concentrations of BHB increased. In sheep, Clarke et al. (1996) found that BHB levels were positively correlated with protein content and negatively correlated with subcutaneous fat depth. These results correspond with conclusions by Van Koevering et al. (1994), that rate of fat deposition can be altered by the inclusion of β-hydroxy-β-methyl butyrate in feedlot diets. Similar to studies in sheep, Richardson et al. (2004) reported positive phenotypic correlations of plasma BHB levels taken from steers at weaning with FCR ($r = 0.36$) and RFI ($r = 0.55$).

Aspartate aminotransferase (AST) and gamma-glutamyltransferase (GGT) are enzymes released from the liver that are used as indicators of liver function. During an infection with liver flukes, migration of juvenile flukes induces inflammation that increases serum AST concentrations (Yang et al., 1998). Aspartate aminotransferase catalyzes the reversible reaction of glutamate and oxaloacetae to aspartate and α-ketoglutyrate. Higher levels of AST indicate higher levels of protein catabolism, which has been reported in cattle with high RFI (Richardson et al., 2004). Liver enzymes are generally poor indicators of nutritional status due to high degree of variation. Gamma-glutamyltransferase catalyzes the extracellular hydrolysis of glutathione and produces cisteinyl-glycine and an enzyme which can react with water to for glutamate. Gamma-glutamyltransferase is mostly used for diagnosis of hepatic diseases such as fasciolosis, a parasitic disease, and the change in liver enzyme concentrations also allows the monitoring of treatment effectiveness. Therefore, there are very few studies that look at GGT and AST in relation to feed efficiency and temperament traits in beef cattle.

Blood urea nitrogen (BUN) is a product of protein degradation, and high BUN concentrations generally indicate excessive mobilization of muscle or a high protein diet. However, high concentrations of BUN have also be shown in cattle fed low protein, low energy diets due to the increase in protein breakdown for energy production. In studies with sheep, Cameron et al. (1992) found negative correlations between BUN and muscle growth and positive correlations with backfat depth. Positive correlations of BUN concentrations have been reported with energy balance and diet supplementation which demonstrates that BUN concentrations can change with dietary quantity and quality of feed (Reist et al., 2002; Chimonyo et al., 2002). Blood urea levels tend to be negatively correlated with feed efficiency and growth rate. The higher concentrations of BUN in less efficient cattle might suggest a greater rate of protein degradation (Richardson et al., 1996).

Proteins circulating in the blood are sources of amino acids for muscle protein synthesis. Total protein (TP) levels have little variability in blood; therefore it can be used as an assessment of nutritional status. Inefficient steers have higher concentrations of TP compared to their more efficient counterparts, which may indicate metabolic differences amongst these two groups (Richardson et al., 1996). First generation steer progeny from divergently selected RFI parents were examined for metabolic processes contributing to variation in RFI, using blood metabolites and blood cell profiles (Richardson and Herd, 2004; Richardson et al., 2004). The results of this study showed significant phenotypic correlations between β-hydroxybutyrate (BHB, $r = 0.55$), aspartate aminotransferase (AST, $r = 0.34$), plasma urea ($r = 0.26$), and total blood

protein (r = 0.26) with RFI at weaning. Heifers with high RFI have been shown to have greater concentrations of BHB and BUN than heifers with low RFI (Kelly et al. 2010, Richardson et al. 1996). Increases in BHB levels, AST concentrations and BUN concentrations may be an indication of an increase in fat breakdown, decrease in protein synthesis and (or) an increase in protein degradation in less efficient cattle.

Blood glucose levels have long been used in metabolic profile tests as the main index of adequate dietary energy. In a study using Mashona cows in Zimbabwe, Chimonyo et al. (2002) looked at the differences in glucose levels of working (pulling drums of water) or non-working cows with and without supplementation (ME = 11.03 MJ/kg). They reported cattle in the supplemented, non-working group to have the highest glucose concentrations and the non-supplemented, working cattle had the lowest, which indicates that glucose is sensitive to dietary changes. Other factors that influence glucose concentrations include physiological status, stress and age. During lactation, cows are in a high energy demand and during stress, an animal's body temperature and respiration rate increase, both of which decrease glucose levels (Ndlovu et al., 2007). A similar reduction is seen in growing cattle where glucose requirement is determined by growth rate rather than maintenance. In conditions such as malnutrition or nutritional stress, precursors derived from the diet decrease causing a decrease in glucose synthesis (Reynolds, 2003). Richardson et al. (2004) conducted a study where plasma samples were taken at weaning, after transport to a feedlot, start and end of feedlot test and found significant positive correlations for glucose levels with ADG (r = 0.57) and RFI (r =

0.40) as well as a negative correlation with FCR (r = -0.46) at the start of the feedlot test period.

Creatinine (CRT) is a product of creatine and phosphocreatine breakdown and has been used as a muscle mass marker in steady state. Variations in creatinine concentrations can be seen due to the animal's diet, muscle mass, and gender (Miller et al., 2004). Clarke et al. (1996) reported creatinine concentrations to have a positive correlation with lean muscle and a negative correlation with fat depth in sheep. In a study with double muscled Belgian Blue bulls, Istasse, et al. (1990) reported strong positive correlations with carcass characteristics (r = 0.97) and a strong negative correlation with adipose tissue (r = -0.87). Similarly, strong negative correlations for creatinine concentrations with RFI were found in beef cattle (Richardson et al., 2004; Richardson and Herd, 2004). These correlations point towards the lower fat content seen in low RFI steers.

Blood cell profiles have also been evaluated as possible sources of variation in RFI. Red blood cell (RBC) counts have shown to be significantly positively correlated with ADG and RFI in British and British crossbred steers (Richardson et al., 2001; Evans and Turner, 1965). However, a study by Theis et al., (2002) found ADG and feed intake to be negatively correlated with RBC counts and hemoglobin (HB) levels at 0 and 70 d and found no correlations for RFI with any blood parameters measured. White blood cell (WBC) counts are often an indicator of disease. Research has shown that during a stress response, the release of glucocorticoids causes a drop in total WBC counts. Richardson et al (2002) reported less efficient steers to have lower WBC counts

which might be due to a perceived stressor. Theis et al., (2002) found no correlation of WBC counts with RFI. Hemoglobin, an iron-containing oxygen-transport protein in red blood cells, has a very high oxygen binding capacity. The main purpose of HB is to carry oxygen from the lungs to the body's tissues and return carbon dioxide from the tissues to the lungs, but it also plays an important role in the shape of RBC. Cattle with low RFI tend to have fewer red blood cells and have lower levels of hemoglobin than cattle with high RFI which may indicate differences in oxygen transport (Richardson et al., 1996). Theis et al., (2002) reported positive correlations for 0 and 70 d serum HB levels and FCR (r = 0.16, 0.25) and negative correlations with feed intake (r = -0.27, -0.34) and ADG (r = -0.34, -0.44). Richardson et al., 2002 found no correlations with blood parameters and RFI but did report significant positive correlations for ADG with RBC (r = 0.16) counts and HB (r = 0.16) levels. In the same study, they reported changes in blood parameters before and after transport and found a significant increase in WBC (13%), RBC (6%) and HB (7%) after transport to a feedlot signifying that a stressor, such as transport, can cause great physiological changes in cattle.

Temperament

Another difference between efficient and non-efficient cattle which has been reported is temperament (Voisinet et al., 1997; Petherick et al., 2002). The term temperament has come to signify many different characteristics such as nervousness, excitability, constitution, or emotionality to name a few. In the beef production industry, temperament is the animal's behavioral response to handling by humans in production scenarios. The first temperament scores were developed by Tulloh (1961) based on a

score ranging from 1 to 6 with the score of 1 considered to be docile or demonstrated minimal reactivity to human handling, and a score of 6 was characterized as an animal with aggressive behavior or demonstrated excessive nervousness in response to human handling. Tulloh (1961) also reported that animals with a low chute score had higher BW than those with a high chute score. In agreement with this study, Voisinet et al. (1997) found that calm animals had a higher ADG than their contemporaries with excitable temperaments. However, they concluded that this was due to calm animals gaining more and not as previously speculated, excitable animals gaining less and consuming less feed. Fell et al. (1999) found that nervous steers had lower ADG for the first 37 d of the study and this lower ADG continued though 78 d of the study.

The negative aspect to chute scores is the subjectivity of the scoring, meaning each handler might score the same animal differently. Eventually this led to Burrow et al. (1988), to develop an objective assessment of temperament. Exit velocity (EV) is defined as the amount of time it takes an animal to traverse a fixed distance of 1.83 m as it exits a confined area (squeeze chute). Cattle with excitable temperaments exit the chute at a faster rate than calm cattle. Brown et al. (2004) found that bull EV at the start of study was negatively correlated with ADG (-0.34) and DMI (-0.25) but not with FCR or RFI. Similar results were reported by Petherick et al. (2002), where exit velocity was significantly correlated with ADG on 0, 21, 45, 70, and 101 d ($r = -0.18, -0.36, -0.32, -.20, -0.25$, respectively) in crossbred steers. In contrast to Brown et al. (2004), a four day average EV was positively correlated with RFI in first generation Angus progeny with parents selected for high and low RFI, such that high efficiency cattle had lower EV

(Richardson et al., 2000). One notable issue with EV is that it changes over time such that EV taken late in studies tends to be slower than those taken at the start of studies. Temperament traits are moderately heritable ranging from 0.07 to 0.44 (Burrow and Corbet, 2000; Burrow, 2001, Hoppe et al., 2010). To further understand the heritability of temperament, Curley et al. (2004) conducted a study using Brahman cattle and concluded that cow temperament ranking (TR; 1- calm, 3 - temperamental) was the best indicator of calf temperament as it was correlated with calf EV ($r = 0.33$), chute score (CS; $r = 0.46$) and pen score (PS; $r = 0.32$). Cow EV was also correlated with calf temperament, but was not as strong as cow temperament ranking.

A recent study looking at animal temperament and stress responsiveness used the average of two temperament indicators (EV and PS) and created a temperament index (King et al., 2006). Animals were then classified as calm (< 1 SD), intermediate (± 1 SD), and excitable (> 1 SD). In this study, the use of pen score was chosen over chute score based on the fact that CS was the least effective in differentiating the animals according to temperament. However, previous studies used chute score as a means of sorting cattle based on temperament and found strong correlations with ADG (Voisinet et al., 1997).

Stress Response

Cattle temperament has also been shown to be associated with stress response. Stress is the body's response to a stimulus. Glucocorticoids are hormones that are released during stress that can compromise the cells of the immune system by interfering with the synthesis and release of cytokines, decrease in lymphocyte proliferation and

antibody production (Blecha and Baker, 1986). Stressed cattle consume less feed, which could further compromise immune function and potentially increase susceptibility to infection (Cole, 1996).

At the onset of a stressor, the biological response is to provide energy substrates for utilization by the animal via the hypothalamic-pituitary adrenal axis (HPA, Knott et al., 2008). The HPA axis stimulates corticotrophin-releasing hormone, which is released from the hypothalamus in the brain. Corticotrophin-releasing hormone acts on the anterior pituitary, which then releases adrenal corticotrophin hormone. Adrenal corticotrophin hormone targets the adrenal glands and cortisol is released from the adrenal cortex. The functions of cortisol include anti-inflammatory activity, blood pressure maintenance, gluconeogenesis, calcium absorption, and the measurement of adrenal cortex function. Cortisol is the primary glucocorticoid involved in stress responses in humans and cattle. Cortisol functions as a stimulus for gluconeogenesis, protein degradation and lipolysis, in order to supply needed energy to the animal during stress. This mobilization of amino acids and fats from cellular reserves is a transfer in nutrient partitioning away from growth to maintenance or energy usage. Cattle with excitable temperaments have higher basal cortisol concentrations than their calmer contemporaries (18.20 vs. 4.30 ng/ml; Curley et al., 2004; Richardson and Herd, 2004). Studies have shown that serum cortisol concentrations and exit velocity are positively correlated ($r = 0.26 – 0.41$) in cattle (Curley et al., 2006, King et al., 2006). Likewise, Fell et al. (1999) reported that nervous cattle examined before weaning, after weaning, and upon entering the feedlot had higher cortisol concentrations and had lower flight

times (sec.) than calm cattle. Theis et al. (2002) reported negative correlations with d 0 cortisol concentrations and ADG r = (-0.24) and feed intake (r = -0.18) in growing steers. When compared with RFI, Richardson et al. (2004) found that steers with low RFI had lower circulating cortisol concentrations after a stress stimulus than steers with high RFI. When a stressor is prolonged, constant a constant release of cortisol concentrations can have a negative effect on ADG and protein accretion which also results in increases of glucose levels, AST concentrations, BUN concentrations and creatinine levels, which indicates muscle membrane damage or protein catabolism (Nockels, 1994). This suggests that in cattle with high RFI, stress might be a source of wasted energy.

CHAPTER II

MATERIALS AND METHODS

Animals and Management

All experimental procedures were in accordance with guidelines for use of Animals in Agricultural Teaching and Research, and approved by the Texas A&M University Institutional Animal Care and Use Committee. A four year post-weaning study was conducted using purebred Brangus heifers from Camp Cooley Ranch (in Franklin, TX) as described by Lancaster et al. (2009). Briefly, each year 114-120 heifers were weaned and placed on a pre-conditioning program at the Camp Cooley Ranch for 25 ± 9 d prior to being transported to the O. D. Butler Jr. Animal Science Teaching Research and Extensions Center in College Station, TX. Upon arrival, heifers were 231.4 ± 11.5 d of age and weighed 271.4 ± 26.1 kg. Heifers were weighed, blocked by BW, randomly assigned to 20 pens (6 head per pen) and adapted to a high roughage diet for 24 d prior to the start of each year's study. Heifers were individually fed using Calan gate feeders twice daily to permit ad libitum feed intake and had free access to water. Individual feed intakes were measured weekly for 70 d. Body weights were taken at 7-d intervals throughout the studies.

Temperament traits were recorded at days 0 and 70 of each year's trial. Chute scores (1-5) were assessed for 30 s after the animal was individually contained in a holding alley prior to entering a working chute. A chute score of 1 was given to heifers that were calm and had no movement in the box where as a chute score of 5 was given to

extremely excitable heifers which tried to escape from the observation box. Exit velocity, the rate at which an animal travels 1.8 meters, was recorded as the animal left the squeeze chute. At the start and end of each year's study, blood samples were collected for analysis via jugular venipuncture using evacuated serum tubes. Samples were allowed to clot overnight at 4ºC and centrifuged at 2000 rpm for 20 min at 4ºC. Serum was harvested and transferred to propylene tubes, frozen and stored at -20ºC until analyzed.

To determine IGF-I concentrations, serum samples were analyzed using an enzyme-linked immunosorbent antibody assay (ELISA) for years 1, 2 and 3. In year 4, concentrations of IGF-I were determined using the radioimmunoassay (RIA) as described by Bilby et al. (1999) with two modifications. The final primary antibody was diluted 1:120,000 and the goat-anti-rabbit secondary antibody was diluted 1:60. The IGF-I antibody used was anti-hIGF-I (AFP4892898, A.F. Parlow, National Hormone and Peptide Program, Torrance, CA). Unknown concentrations of IGF-I were calculated using Assay Zap software (Biosoft, Cambridge, UK) using counts per minute (cpm) obtained from a Cobra II auto-gamma-counter (Perkin Elmer, Waltham, MA).

In all four years of the study, serum cortisol levels were examined using the same assay. The Coat-A-Count Cortisol assay is a solid phase radioimmunoassay which uses ^{125}I-labeld cortisol to competitively exclude native serum cortisol for antibody sites on the wall of a polypropylene tube, for a fixed amount of time. Unknown concentrations of cortisol were calculated using a calibration curve derived from the Assay Zap software

(Biosoft, Cambridge, UK) using counts per minute (cpm) obtained from a Cobra II auto-gamma-counter (Perkin Elmer, Waltham, MA).

Serum samples were sent to an independent lab (Texas Veterinary Medical Diagnostic Laboratory System) for analysis of partial blood counts and a ruminant chemistry panel (College Station, TX) and beta-hydroxybutyrate (BHB) using an Olympus AU4E Chemistry Analyzer (Amarillo, TX). Partial blood count consisted of red blood cell count (RBC), white blood cell count (WBC), and hemoglobin (HB), and the chemistry panel included serum total protein (TP), glucose, blood urea nitrogen (BUN), creatinine, aspartate transaminase (AST), and gamma-glutamyltransferase (GGT).

Diet ingredient samples were collected weekly and composited by weight at the end of each year's trial. Ingredients were dried in a forced air oven at 105 ºC and ground through a Wiley Mill to pass a 1 mm screen. Composited ingredients were sent to an independent lab (Cumberland Valley Analytical Services, Inc., Hagerstown, MD) for chemical analysis. Metabolizable energy concentrations of the diets were computed from the chemical analysis using the Cornell Net Carbohydrate and Protein System (Version 5.0, Cornell University, Ithaca, NY). Ingredient and chemical composition of each year's diets are presented in Tables 2.1 and 2.2, correspondingly.

Computation of Traits

Individual heifer growth rates were modeled by linear regression of BW on day of trial using PROC GLM of SAS (SAS Inst., Cary, NC) and regression coefficients were used to calculate ADG, initial BW, final BW, and metabolic BW (MBW; mid-test

Table 2.1 Ingredient composition of the diet fed to Brangus heifers in all four trials

Ingredient	Value, as-fed %
Chopped alfalfa hay	35.00
Pelleted alfalfa	15.00
Dry rolled corn	20.95
Cottonseed hulls	21.50
Molasses	7.00
Salt	0.40
Vitamin E[1]	0.14
Trace mineral[2]	0.02

[1]Vitamin E contained 44,000 IU/kg of product.

[2]Trace mineral contained minimum 19.0% Zn, 7.0% Mn, 4.5% CU, 4,000 ppm Fe, 2,300 ppm I, 1,000 ppm Se and 500 ppm Co.

Table 2.2 Chemical composition of the diet fed Brangus heifers in each of the four trials

Item	Trial 1	Trial 2	Trial 3	Trial 4
DM%	87.49	89.36	88.05	86.81
CP, % DM	12.57	13.16	12.48	12.58
NDF, % DM	43.04	43.75	44.97	45.87
ME, Mcal/kg DM[1]	2.03	2.00	1.93	1.96

[1]Metabolizable energy content computed using Cornell Net Carbohydrate and Protein System.

$BW^{.75}$). Moisture analyses of diet ingredient samples were used to compute average daily DMI from feed intake data. Residual feed intake (RFIp) was computed within each trial by actual DMI minus predicted DMI to meet growth and maintenance energy requirements (Koch et al., 1963).

Expected DMI was calculated from the linear regression of DMI on ADG and MBW with trial and trial by independent variable interactions as random effects. Feed conversion ratio (FCR) was calculated as the ratio of DMI and ADG.

To determine if individual animal variation in temperament traits and serum hormones and metabolites affected the derivation of expected DMI, a 2 step approach was employed (Arthur et al., 2003; Lancaster et al., 2009a). Initially, stepwise regression analysis was performed (PROC REG of SAS) to determine the order in which 1) temperament traits or 2) serum hormones and serum metabolites would be added to the base model of RFI which includes MBW and ADG. Subsequently, the order of significant traits added to the base model and the resulting change in R^2 was used to determine their relative importance to account for additional variation in DMI.

Based on previous research by Lancaster et al. (2009a), three models were used to evaluate the differences in combining data from multiple trials and the inclusion of temperament and serum hormones and metabolites, to compute.

Model 1 regressed DMI on MBW, ADG and temperament traits or serum parameters with the inclusion of trial as a fixed effect.

$$Y_{ij} = ß_0 + ß_1MBW_{ij} + ß_2ADG_{ij} + ß_3T_i + ß_xX_{ijk} + e_{ij}$$

Where Yij is the DMI of the *j*th heifer in the *i*th trial, T_i is the fixed effect of the *i*th trial, X_{ijk} is the *k*th temperament trait or serum parameter for the *j*th heifer in the *i*th trial, $ß_0$ is the regression intercept, $ß_1$ is the regression coefficient of MBW, $ß_2$ is the regression coefficient of ADG, $ß_3$ is the regression coefficient on trial, β_x is the regression coefficient on temperament trait or serum parameter X, and e_{ij} is the random uncontrolled error and error associated with fixed interactions of independent variables and trial for the *j*th heifer in the *i*th trial.

Model 2 regressed DMI on MBW, ADG and temperament traits or serum parameters with the inclusion of trial and trial by independent variable interactions as random effects to account for potential variation in mean DMI and the differential relationships of DMI with MBW, ADG and temperament traits or serum parameters due to trial.

$$Y_{ij} = \beta_0 + \beta_1 MBW_{ij} + \beta_2 ADG_{ij} + \beta_3 \tau_i + (\beta_4 MBW_j * \tau_i) + (\beta_5 ADG_j * \tau_i) + \beta_{x1} X_{ijk} + (\beta_{x2} X_{jk} * \tau_i) + e_{ij}$$

Where Y_{ij} is the DMI of the *j*th heifer in the *i*th trial, τ_i is the random effect of the *i*th trial, X_{ijk} is the *k*th temperament trait or serum parameter for the *j*th heifer in the *i*th trial, β_0 is the regression intercept, β_1 is the regression coefficient on MBW, β_2 is the regression coefficient on ADG, β_3 is the regression coefficient on random trial, β_4 is the regression coefficient on the random interaction of MBW and trial, β_5 is the regression coefficient on the random interaction of ADG and trial, β_{x1} is

the regression coefficient on the *k*th temperament trait or serum parameter, β_{x2} is the regression coefficient on the random interaction of *k*th temperament trait or serum parameter and *i*th trial, and e_{ij} is the uncontrolled error for the *j*th heifer in the *i*th trial.

Model 3 was similar to Model 2 in that it includes trial and trial by independent variable interactions as random effects however, to compute the R^2, an adjusted DMI trait was computed using only the fixed effects and the residual from the complete model, and then regressed on MBW and ADG.

$$Y_j^* = \beta_0 + \beta_1 MBW_j + \beta_2 ADG_j + \beta_{x1} X_j + e_j$$

Where Y_j^* is the DMI of the *j*th heifer without the random effect of the *i*th trial and was computed as $Y_{ij} - [\beta_3\tau_i + (\beta_4 MBW_j^* \tau_i) + (\beta_5 ADG_j^* \tau_i) + (\beta_{x2} X_j^* \tau_i)]$, Y_{ij} is the DMI of the *j*th heifer in the *i*th trial, τ_i is the random effect of the *i*th trial, X_j is the temperament trait or serum parameter for the *j*th heifer, β_0 is the regression intercept, β_1 is the regression coefficient on MBW, β_2 is the regression coefficient on ADG, β_3 is the regression coefficient on random trial, β_4 is the regression tcoefficient on the random interaction of MBW and trial, β_5 is the regression coefficient on the random interaction of ADG and trial, β_{x1} is the regression coefficient on the temperament trait or serum parameter X, β_{x2} is the regression coefficient on the random interaction of temperament trait or serum parameter X and e_{ij} is the uncontrolled error for the *j*th heifer in the *i*th trial.

The R^2 was compared amongst the three models by testing the extra sums of squares (Neter et al., 1996). Results from these three analyses were used to evaluate the inclusion of temperament traits and serum parameters to calculate expected DMI.

Exit velocity was adjusted by taking the difference of the mean EV for each year from each animal's EV and divided by the mean EV to find a relative exit velocity (REV). A relative chute score (RCS) was calculated using the same method. A temperament index (TI) was calculated by taking the average of REV and RCS for each individual heifer, (King et al., 2006).

Statistical Analysis

All animal performance, feed efficiency traits, temperament traits, serum hormone, and metabolite values were adjusted to remove the random effect of year using the PROC MIXED of SAS (SAS Inst., Cary, NC). PROC CORR was used to examine the phenotypic correlations between RFI, adjusted performance traits, adjusted temperament traits, and adjusted serum hormones and metabolites. Heifers were then classified into low, medium, and high RFI groups that were < 0.5, ± 0.5, and > 0.5 SD, respectively, from the mean RFIp of 0.00 ± 0.71 kg/d. Least squares procedures using the MIXED procedure of SAS were used to examine the effects of RFIp group on performance, feed efficiency, temperament, serum hormones, and serum metabolites. Comparisons of least square means between RFIp groups were performed using Tukey's post hoc test. Heifers were also classified into low, medium, and high temperament index (TI) groups that were < 0.5, ± 0.5, and > 0.5 SD, respectively, from the mean TI of 0.00 ± 0.25. Least squares procedures using the MIXED procedure of SAS were used to

examine the effects of TI group on performance, feed efficiency, serum hormones, and serum metabolites. Comparisons of least square means between TI groups were performed using Tukey's post hoc test.

CHAPTER III

RESULTS AND CONCLUSIONS

Performance and Feed Efficiency Statistics

Summary statistics of performance and feed efficiency traits for all four trials are presented in Table 3.1 for each of the 4 trials. Average initial age of the heifers (n = 468) was 231 ± 12 d and ranged from 226 ± 9 d in year 1 to 236 ± 15 d in year 3. The overall summary statistics adjusted for random effects of trial are presented in Table 3.2. Heifers had an initial BW which averaged 271 ± 26 kg. All heifers in the 4 trials averaged 1.01 kg/d (range 0.59 to 1.53 kg/d) for ADG, 9.45 kg/d (range 6.87 to 12.56 kg/d) for DMI, and 9.49 kg DM/ kg of gain (range 6.67 to 15.65) for FCR. Previous work by Arthur et al (2003) reported similar means and SD for ADG (1.19 ± 0.19 kg/d) and DMI (9.2 ± 1.2 kg/d) in Angus heifers fed a high roughage diet with a similar ME concentration of 2.5 Mcal/kg. Mean phenotypic RFI was 0.00 ± 0.71 kg/d and ranged from -2.02 to 2.16 kg/d. The phenotypic SD for RFIp in this study was similar to the SD reported in previous studies (0.60, 0.74, 0.76; Lancaster et al., 2009a, Arthur et al., 2001a, Arthur et al., 2001b, respectively). Baker et al. (2006) reported a mean RFI 0.00 ± 0.48 kg/d with ultrasound fat thickness included in the DMI model and Basarab et al. (2003) reported a mean RFI of 0.00 ± 0.60 kg/d with back fat gain included in the DMI model; both of which are similar to the RFIc in this study (0.00 ± 0.69 kg/d).

Table 3.1 Summary statistics (± SE) of performance and feed efficiency traits for Brangus heifers in each of the four trials

Trait[1]	Trial 1	Trial 2	Trial 3	Trial 4
No. animals	114	115	119	120
Initial age, d	225.8 ± 9.1	236.0 ± 10.7	235.6 ± 14.6	228.3 ± 11.7
Initial BW, kg	285.1 ± 28.0	268.5 ± 23.8	267.8 ± 25.8	264.4 ± 26.9
Final BW, kg	345.8 ± 31.2	342.9 ± 28.9	377.7 ± 29.0	339.7 ± 30.0
Mid-test $BW^{.75}$, kg	75.01 ± 5.18	73.06 ± 4.65	70.63 ± 4.84	72.40 ± 5.02
ADG, kg/d	0.90 ± 0.15	1.06 ± 0.16	1.00 ± 0.13	1.08 ± 0.17
DMI, kg/d	9.10 ± 1.11	9.47 ± 1.04	9.71 ± 1.04	9.53 ± 0.88
FCR, DMI/gain	10.26 ± 1.54	9.04 ± 1.31	9.80 ± 0.97	9.01 ± 1.21
RFIp, kg/d	0.00 ± 0.75	0.00 ± 0.68	0.00 ± 0.68	0.00 ± 0.66
RFIc, kg/d	-0.02 ± 0.75	0.00 ± 0.67	0.00 ± 0.69	0.02 ± 0.68

[1]MBW = mid-test $BW^{0.75}$; FCR = feed conversion ration; RFIp = residual feed intake from base model; RFIc = residual feed intake from carcass-adjusted model.

Table 3.2 Overall summary statistics of trial adjusted performance and feed efficiency traits of Brangus heifers

Trait[1]	Mean	SD	Min	Max
Initial age, d	231.4	11.7	197.0	259.0
Initial BW, kg	271.4	26.1	211.1	337.9
Final BW, kg	341.5	29.7	273.5	421.3
Mid-test $BW^{.75}$, kg	72.77	4.91	61.48	85.33
ADG, kg/d	1.01	0.15	0.59	1.53
DMI, kg/d	9.45	1.02	6.87	12.56
FCR, DMI/gain	9.49	1.27	6.67	15.65
RFIp, kg/d	0.00	0.71	-2.02	2.16
RFIc, kg/d	0.00	0.69	-1.90	1.98

[1]MBW = mid-test $BW^{0.75}$; FCR = feed conversion ration; RFIp = residual feed intake from base model; RFIc = residual feed intake from carcass-adjusted model.

Temperament and Serum Metabolites Statistics

Overall summary statistics of temperament and trial adjusted serum metabolites are reported on Table 3.3. Mean adjusted serum IGF-I was 129.6 ± 33.3 ng/mL which was similar to the mean reported by Davis and Simmen (1997; 182.0 ng/mL) and Lancaster et al. (2008; 138.0 ng/mL). Mean partial blood counts were 10.01 ± 1.0 $x10^6/\mu L$, 9.95 ± 2.4 $x10^3/\mu L$ and 12.82 ± 1.1 g/dL for RBC, WBC and HB, respectively. Richardson et al. (2002) reported lower numbers for RBC and WBC and higher concentrations for HB. However, the mean values for this study were within the reference ranges of 5.0 to 10.0 $x10^6/\mu L$ for RBC, 4 to 12 $x10^3/\mu L$ for WBC and 8.5 to 15.0 g/dL for HB (Texas Veterinary Medical Diagnostic Laboratory System, TVMDL). The mean value for TP was 6.33 ± 1.1 g/dL which is similar to values reported by Richardson et al. (1996, 2004) and was within the acceptable range of 6.2 to 9.3 g/dL (TVMDL). Blood urea nitrogen has a reference range of 10 to 25 mg/dL (TVMDL) which includes the mean of 11.11 ± 1.8 mg/dL. The reference concentration for BHB ranges from 120 to 610 μmol/L (Agenas et al., 2006) which encompasses the mean BHB (254.3 ± 69.4 μmol/L) for this study. Beta-hydroxybutyrate concentrations were similar to those reported by Richardson et al. (2004) of 265.0 μmol/L. The mean of glucose was 92.54 ± 19.1 mg/dL, which falls within the reference range of 45 to 102 mg/dl (TVMDL), and was similar to the mean glucose levels (93.8 mg/dL) reported by Kolath et al.(2006). Creatinine concentrations range from 0.5 to 1.7 mg/dL (TVMDL) and the mean from this study was 1.08 ± 0.2mg/dL. Mean liver enzyme concentrations for GGT and AST were 14.66 ± 5.6 and 21.75 ± 13.5. Gamma-glutamyltransferase

Table 3.3 Overall summary statistics of trial adjusted temperament and serum metabolites of Brangus heifers

Trait[1]	Mean	SD	Min	Max
Initial relative exit velocity	0.00	0.25	-0.66	0.83
Initial relative chute score	0.00	0.39	-0.54	2.56
Initial temperament index	0.00	0.25	-0.55	1.35
IGF-I, ng/mL	129.6	33.3	42.9	247.4
Cortisol, ng/mL	4.53	1.82	0.62	9.28
RBC, x 10^6/μL	10.01	1.00	6.63	13.53
WBC, x 10^3/μL	9.95	2.42	4.42	18.93
Hemoglobin, g/dL	12.82	1.05	9.36	16.16
Total protein, g/dL	6.33	1.10	2.19	7.99
Blood urea nitrogen, mg/dL	11.11	1.82	6.43	16.99
BHB, μmol/L	254.3	69.4	22.2	540.5
Glucose, mg/dL	92.54	19.14	17.42	236.4
Creatinine, mg/dL	1.08	0.16	0.10	1.89
GGT, U/L	14.66	5.56	-0.23	49.44
AST, U/L	21.75	13.50	-3.37	69.98

[1]IGF-I = insulin-like growth factor-I; RBC = red blood cell; WBC = white blood cell; BHB = beta-hydroxybutyrate; GGT = gamma-glutamyltransferase; AST = aspartate transaminase

concentrations were higher than those reported by Richardson et al. (2004; 11.0 U/L) yet fell within the reference range of 11 to 39 U/L (TVMDL); however, the mean AST concentration was significantly lower than those reported by Richardson et al. (2004; 63.7 U/L) reported and did not fall within the ranges of 47 to 138 U/L (TVMDL).

Performance Traits and Feed Efficiency

Phenotypic correlations for performance and feed efficiency traits are reported in Table 3.4. Initial and final BW were moderately to highly correlated with ADG (r = 0.11, 0.35, respectively) and DMI (r = 0.38, 0.50, respectively). As expected, strong correlations between ADG and DMI (r = 0.57) and FCR (r = -0.71) were found. Similar to our findings, Lancaster et al. (2009a) and Arthur et al. (2001a, 2001b) reported correlations for ADG with DMI (r = 0.66, 0.41, 0.47, respectively), and FCR (r = -0.72, -0.74, -0.54, respectively). Dry matter intake was positively correlated with FCR (r = 0.15), which is consistent with phenotypic correlations reported by Nkrumah et al, (2007) and Lancaster et al. (2009a). As expected, RFIp and RFIc were not correlated with growth traits. However, RFIp and RFIc were strongly correlated with DMI (r = 0.70 and 0.67, respectively) and FCR (r = 0.59 and 0.58, respectively). These results indicate that selection for RFI will result in a more favorable FCR. Correlations between RFIp and RFIc were strong (r = 0.97) indicating that selection for RFIc would also result in a more favorable FCR.

Feed Efficiency and Temperament

Previous research has shown that animal temperament behavior affects cattle performance in a negative manner (Richardson et al., 2000). Voisinet et al. (1997) found

Table 3.4 Phenotypic correlations among trial adjusted performance and feed efficiency traits of Brangus heifers

Trait[1]	ADG	DMI	FCR	RFIp	RFIc
Initial BW, kg	0.11*	0.38*	0.19*	0.01	0.01
Final BW, kg	0.35*	0.50*	0.00	0.03	0.03
Mid-test $BW^{.75}$, kg	0.33*	0.59*	0.10*	0.00	0.01
ADG, kg/d		0.57*	-0.71*	-0.01	-0.01
DMI, kg/d			0.15*	0.70*	0.67*
FCR, DMI/gain				0.59*	0.58*
RFIp, kg/d					0.97*

[1]MBW = mid-test $BW^{0.75}$; FCR = feed conversion ration; RFIp = residual feed intake from base model; RFIc = residual feed intake from carcass-adjusted model.

*Correlations are different from zero at $P < 0.05$.

that cattle with calmer temperaments had 14% higher ADG than cattle with excitable temperaments. Phenotypic correlations for temperament traits with performance and efficiency traits are reported on Table 3.5. Brown et al., (2004) reported EV to be negatively correlated with ADG, and DMI (r = -0.25, and -0.17). Similar observations have been reported regarding negative correlations with EV and ADG, such that as EV increased (more temperamental), ADG decreased (Petherick et al. 2002; 2003). In this study, initial REV was negatively correlated with ADG and DMI (r = -0.16, -0.21) but not with FCR or RFIp. Hoppe et al, (2010) reported negative correlations with CS and ADG across various breeds of cattle and found they ranged from -0.13 to -0.58. Initial RCS was negatively correlated with ADG (r = -0.13) and positively correlated with FCR (r = 0.10). Initial temperament index was significantly correlated with ADG and DMI (r = -0.18, and -0.16, respectively).

Appendix A reports weaning, 0 d and 70 d exit velocity correlations with performance and feed efficiency traits. Wean REV, Initial REV, and Final REV were negatively correlated with Final BW (r = -0.11, -0.13, -0.15, respectively) and DMI (r = -0.12, -0.21, -0.19 respectively). Initial and final EV were also negatively correlated with ADG (-0.16 and -0.17, respectively) which indicates that excitable heifers (high REV) throughout the 70 d study had lower BW, DMI and ADG than calmer heifers.

Feed Efficiency and Serum Metabolites

Based on research completed in Australia, the Australian Angus Association incorporated the use of IGF-I as an indicator trait for genetic evaluation of RFI. Johnston et al. (2001) and Moore et al. (2005) reported positive genetic correlations

Table 3.5 Phenotypic correlations among trial adjusted performance and feed efficiency traits with trail adjusted temperament and serum metabolites of Brangus heifers

Trait[1]	ADG[2]	DMI	FCR	RFIp	RFIc
Initial relative exit velocity	-0.16*	-0.21*	0.01	-0.05	-0.03
Initial relative chute score	-0.13*	-0.07	0.10*	0.00	0.03
Initial temperament index	-0.18*	-0.16*	0.08	-0.02	0.01
IGF-I, ng/mL	0.02	0.08	0.04	-0.01	-0.01
Cortisol, ng/mL	-0.02	-0.08	-0.03	-0.02	0.01
RBC, x 10^6/μL	-0.07	-0.11*	-0.01	-0.07	-0.04
WBC, x 10^3/μL	0.01	0.10*	0.09	0.15*	0.15*
Hemoglobin, g/dL	-0.19*	-0.18*	0.08	-0.11*	-0.09*
Total protein, g/dL	0.04	-0.06	-0.09	-0.10*	-0.09
Blood urea nitrogen, mg/dL	-0.13*	0.08	0.19*	0.10*	0.06
BHB, μmol/L	0.11*	0.18*	0.02	0.13*	0.07
Glucose, mg/dL	-0.25*	-0.19*	0.16*	0.00	0.02
Creatinine, mg/dL	-0.10*	-0.07	0.06	-0.11*	-0.12*
GGT, U/L	-0.06	-0.04	0.06	-0.03	0.00
AST, U/L	0.01	0.03	0.04	0.05	0.09

[1]IGF-I = insulin-like growth factor-I; RBC = red blood cell; WBC = white blood cell; BHB = beta-hydroxybutyrate; GGT = gamma-glutamyltransferase; AST = aspartate transaminase.

[2]FCR = feed conversion ration; RFIp = residual feed intake from base model; RFIc = residual feed intake from carcass-adjusted model.

*Correlations are different from zero at $P < 0.05$.

between post weaning IGF-I concentrations and RFI in *Bos taurus* cattle (r_g = 0.39 and 0.57, respectively). The moderate to high heritability estimates for IGF-I (0.25 – 0.68) allows IGF-I to be a potential biological indicator for RFI (Davis and Bishop, 1991; Davis and Simmen, 2006). However, no phenotypic correlations were seen between RFI and serum hormone IGF-I concentrations (Table 3.5). Similarly, Kelly et al. (2010) and Richardson et al. (1996) found no significant relationship between RFI and IGF-I in growing steers. Serum IGF-I was not correlated with any other performance or feed efficiency traits, which is in contrast to results reported by Kelly et al. (2010) who reported that serum IGF-I was correlated with ADG (r = 0.26) and Brown et al. (2004) who reported correlations with FCR (r = 0.36).

Fell et al., (1999) reported significant negative correlations for post weaning cortisol and feedlot entry cortisol concentrations with ADG (r = -0.57 and -0.54, respectively). In contrast to our study, Theis et al. (2002) found correlations for cortisol with ADG (r = -0.24), DMI (r = -0.18) and FCR (r = 0.16) in crossbred steers. Richardson and Herd (2004) found a very weak correlation for RFI and cortisol (r = 0.005) in steers which is in contrast to this study and Theis et al. (2002). In rams, Knott et al. (2004) reported a significant positive correlation with RFI and pre-stress challenge and post-stress challenge cortisol concentrations (r = 0.23 and 0.65). In this study, cortisol concentrations were found to have no phenotypic correlations with any performance or feed efficiency traits.

Results from partial blood counts found WBC counts to be correlated with RFIp and RFIc (r =0.15, and 0.15). Richardson et al. (2002) reported less efficient steers as

having lower WBC counts which might be indicative of stress in these high RFI cattle; however, this study found positive correlations with RFI and WBC counts. Hemoglobin was negatively correlated with ADG (r = -0.19), DMI (r = -0.18), RFIp (r = -0.11) and RFIc (r = -0.09). This negative correlation with HB indicates that heifers with low RFI have higher HB in their circulating blood which would allow for a higher oxygen carrying capacity. In agreement with our findings, Theis et al. (2002) reported significant negative correlations for 0 d HB concentrations with ADG and DMI (r = -0.34 and -0.27, respectively). Theis et al. (2002) also reported negative correlations for RBC counts with ADG and DMI (r = -0.19 and -0.27, respectively) which is similar to the findings of this study (r = -0.07 and -0.11, respectively).

Weaning BUN concentrations previously reported to be positively correlated (r = 0.26) with RFI but not at any other time point in the approximately 180 d study (Richardson et al., 2004). Kelly et al., (2010) found positive correlations for BUN concentrations with DMI and FCR (r = 0.46 and 0.42) over the whole 82 d study. These studies are in agreement with the findings from this study where BUN concentrations showed to be correlated with ADG (r = -0.13), FCR (r = 0.19) and RFIp (r = 0.10). This positive relationship with RFI explains a greater rate of amino acid catabolism in animals with high RFI as reported by Richardson et al. (2004). Total protein was negatively correlated with RFIp (r = -0.10) which would suggest that more efficient animals have higher levels of TP which is contrary to the findings by Richardson et al. (2004) who found a tendency for a positive correlation for TP with RFI.

Beta-hydroxybutyrate concentrations were correlated with all performance traits (ADG, 0.11; DMI, 0.18; and RFIp, 0.13) except FCR and RFIc. Higher correlation coefficients were reported by Richardson and Herd (2004), Richardson et al. (2004), and Kelly et al. (2010) for BHB concentrations and RFI (r = 0.24, 0.55, and 0.37 respectively). Cattle with high RFI have been reported to have higher stress responses than cattle with low RFI which might explain the differences in BHB concentrations since it is a product of lipolysis and under stress, the body increases available energy by breaking down fat. Similar to Kelly et al. (2010) and Richardson et al. (2004), no correlations existed between glucose and RFI. However, glucose was correlated with ADG (r = -0.25), DMI (r = -0.19), and FCR (r = 0.16) which is in contrast to the two aforementioned studies that reported no significant association for glucose with ADG, DMI, and FCR. Weak correlations were found for creatinine with ADG (r = -0.10) and RFIp (r = -0.11) which likely reflects the greater muscle mass of heifers with low RFI. The RFI and creatinine correlation coefficient was much lower in this study compared to Richardson and Herd (2004) and Richardson et al. (2004; r = -0.30 and -0.45, respectively). These two studies also reported positive correlations for AST levels and RFI (r = 0.34 and 0.25, respectively) whereas; no correlations were seen with any performance or feed efficiency traits and liver enzyme concentrations (AST and GGT) in this study.

Divergent RFI Groups

Least square means for performance and feed efficiency traits for heifers with divergent RFIp are presented in Table 3.6. Heifers with low RFI had significantly lower

DMI (8.69 vs. 10.29 kg/d) and FCR (8.70 vs. 10.36) than heifers with high RFI. Kelly et al. (2010) reported a 15.9% difference in DMI between beef heifers with low and high RFI. Growing steers studies have also seen a difference in DMI between divergent RFI groups, ranging from 5% to 16% (Richardson et al., 2004; Richardson and Herd, 2004; Lancaster et al., 2009a). As expected, there was no difference in initial BW or ADG amongst heifers with divergent RFI.

Differences in temperament and serum metabolites amongst divergent RFI groups are reported on Table 3.7. Temperament traits did not differ amongst RFI groups. There were also no differences in IGF-I (P = 0.64), cortisol (P = 0.76), total protein (P =.20), glucose (P = 0.79) AST (P = 0.39) and GGT (P = 0.96) in divergent RFI groups. Significant differences were seen amongst low vs. high RFI groups for WBC counts (9.48 vs.10.50 x 10^6/μL) such that heifers with low RFI had 10% lower WBC counts than heifers with high RFI. This is in contrast to research reported by Richardson et al. (2002) that found significantly higher WBC counts in steers with low RFI. In this current study, hemoglobin concentrations were higher for heifers with low RFI (13.00 vs. 12.68 kg/d) than high RFI heifers. This is in contrast to previous studies by Richardson et al. (2000; 2002) that saw 4% and 3%, respectively, lower levels of HB concentrations in steers with low RFI.

Heifers with low RFI had 5% lower levels of BUN concentrations compared to high RFI heifers. Similar to our findings, Richardson et al. (2004) reported that high RFI steers have a higher rate of protein degradation which can contribute to the positive

Table 3.6 Trial adjusted performance and feed efficiency traits for Brangus heifers with divergent RFIp phenotypes[1]

Trait[2]	Low-RFIp	High-RFIp	SE	P-Value
No. of heifers	150	142	-	-
Initial BW, kg	271.1	270.5	3.1	0.85
Final BW, kg	340.4	341.0	3.5	0.87
MBW, $kg^{0.75}$	72.88	72.69	0.58	0.74
ADG, kg/d	1.01	1.01	0.02	0.98
DMI, kg/d	8.69	10.29	0.09	0.001
FCR, DMI/gain	8.70	10.36	0.13	0.001
RFIp, kg/d	-0.77	0.83	0.04	0.001
RFIc, kg/d	-0.75	0.78	0.04	0.001

[1]Heifers with low and high RFIp were < 0.5 SD and > 0.5 SD from the mean RFI of 0.00 ± 0.71, respectively.

[2]MBW = mid-test $BW^{0.75}$; FCR = feed conversion ration; RFIp = residual feed intake from base model; RFIc = residual feed intake from carcass-adjusted model.

Table 3.7 Trial adjusted temperament traits and serum metabolites for Brangus heifers with divergent RFIp phenotypes[1]

Trait[2]	Low-RFI	High-RFI	SE	P-Value
Initial relative exit velocity	0.02	-0.02	0.03	0.21
Initial relative chute score	0.02	0.02	0.05	0.93
Initial temperament index	0.02	0.00	0.03	0.48
IGF-I, ng/mL	130.5	128.6	3.9	0.64
Cortisol, ng/mL	4.46	4.52	0.21	0.76
RBC, x 10^6/μL	10.11	9.94	0.12	0.16
WBC, x 10^3/μL	9.48	10.50	0.28	0.001
Hemoglobin, g/dL	13.00	12.68	0.12	0.01
Total protein, g/dL	6.15	6.00	0.12	0.20
Blood urea nitrogen, mg/dL	10.69	11.23	0.21	0.01
BHB, μmol/L	248.2	267.0	8.1	0.02
Glucose, mg/dL	92.73	93.33	2.25	0.79
Creatinine, mg/dL	1.11	1.07	0.02	0.06
GGT, U/L	13.45	13.42	0.64	0.96
AST, U/L	24.13	25.45	1.52	0.39

[1]Heifers with low and high RFIp were < 0.5 SD and > 0.5 SD from the mean RFI of 0.00 ± 0.71, respectively.

[2] IGF-I = insulin-like growth factor-I; RBC = red blood cell; WBC = white blood cell; BHB = beta-hydroxybutyrate; GGT = gamma-glutamyltransferase; AST = aspartate transaminase.

association with BUN levels. However, there has been a lack of consistency among studies reporting BUN level correlations with RFI, which might be explained by differences in body composition of the animal and feed intake levels amongst RFI groups. Beta-hydroxybutyrate levels were 7% lower in heifers with low RFI (248.23 μmol/L) compared to heifers with high RFI (267.02 μmol/L). Even though Richardson et al. (2004) saw a strong positive correlation (r = 0.55) with BHB and RFI, they didn't see any significant differences amongst RFI groups.

Model Variation in the Prediction of DMI

The amount of variation explained by the base model which regresses DMI on MBW and ADG is presented in Table 3.8. As reported by Lancaster et al. (2009b), the base regression explained 47% of the variation in DMI. The R^2 for each individual trial ranged from 0.433 to 0.573 and averaged 0.529 for the base model. Lancaster et al. (2009b) concluded that Model 2, which included trial and trial by independent variables interactions as random effects, provided the best estimate of RFI when calculated across multiple trails and Model 3, which removes the random effects from actual DMI to obtain DMI adjusted by test, best describes the variation in DMI explained by ADG and MBW since it presents an estimation of variation similar to the average of the four trail's base model R^2.

Table 3.8 Percentage of variance (R^2) in feed intake explained by inclusion of serum parameters and ultrasound carcass traits

	Model Number[1]		
Regression[2]	$F2+e_1$	$F1+R+e_2$	$(F1+R)-R+e_3$
Base model (BM; ADG and MBW)	0.472	0.538	0.531
BM + carcass traits	0.558	0.562	0.551
BM + serum parameters	0.542	0.595	0.589
BM + serum and carcass traits	0.571	0.625	0.618

[1] F1= fixed effects of indicated variables; F2 = fixed effects of indicated variables + fixed effect of test; R = random effects of test and test by independent variable interactions; e_1 = random uncontrolled error and error associated with fixed interactions of test and independent variables; e_2 = random uncontrolled error; e_3 = random uncontrolled error.

[2] Carcass traits = gain in BF, final LMA; Serum parameters = blood urea nitrogen, creatinine, white blood cells, total protein, glucose, hemoglobin and beta-hydroxybutyrate.

The R^2 for the base model is significantly lower (0.53) than those previously reported which range from 0.68 to 0.82 (Basarab et al., 2003; Baker et al., 2006; Lancaster et al. 2009a). Lancaster et al. (2009b) concluded that the higher R^2 was likely due to diet and gender of cattle. These previous studies used a high energy grain based diet and used bulls and steers where as this study fed a low energy roughage based diet to heifers.

Residual feed intake adjusted for carcass traits was calculated from the regression model for DMI with gain in back fat and final longissimus muscle area in addition to MBW and ADG (Lancaster et al., 2009b). Basarab et al. (2003) and Baker et al. (2006) included carcass fat traits (fat gain and ultrasound fat thickness, respectively) in the regression model for DMI and reported R^2 ranging from 0.71 to 0.85 which is also higher than the R^2 reported in this study (0.551).

The order of inclusion of serum metabolites was determined from the results of stepwise regression analysis which included blood urea nitrogen (BUN), creatinine (CRT), white blood cell counts (WBC), total protein (TP), glucose (GLUC), hemoglobin (HB), and beta-hydroxybutyrate (BHB). The amount of additional variation explained over the base model is presented in Table 3.8. Of these seven variables, WBC had the highest R^2 (0.542) and therefore explained the largest amount of additional variation (2.4%) according to Model 4 (data presented in Appendix B). Including the 6 remaining serum metabolites increased the R^2 to 0.589 which accounts for 12.6% of the additional variation in DMI. The addition of serum metabolites to the BM with carcass traits

resulted in the R^2 increasing from 0.551 to 0.618 which explains 15.4% of the variation in DMI.

Temperament traits were also run through stepwise regression analysis to determine the order of inclusion of the variables that might account for additional variation in DMI. The only trait included was average relative exit velocity (AvgREV) which is the average of wean REV, 0 d REV, and 70 d REV for each heifer. The inclusion of AvgREV raised the R^2 0.1 percentage point (data presented in Appendix B). Since temperament traits accounted for very little variation in DMI, it was removed from the models.

Temperament and Serum Metabolites

It has been established that animal temperament and stress have significant effects on blood metabolite concentrations. However, few studies have looked specifically at the effect of EV on serum metabolite concentrations, with the exception of cortisol, in beef cattle. Phenotypic correlations among temperament traits and serum metabolite concentrations are presented in Table 3.9. Previous studies have reported significant positive correlations with temperament and cortisol concentrations (Stahringer et al., 1990; Fell et al., 1999; Curley et al., 2004). In this study, Initial REV, Initial RCS and initial TI were positively correlated with cortisol (r = 0.22, 0.23 and 0.29, respectively). Curley et al. (2006) saw similar correlations with EV (r = 0.26) but not chute score (r = 0.09). The three temperament traits were also negatively correlated with BHB (r = -0.15, -0.22, -0.25, respectively) which suggests that as heifers became

Table 3.9 Phenotypic correlations among trial adjusted temperament traits with trial adjusted serum metabolites in Brangus heifers

Trait[1]	Initial REV	Initial RCS	Initial TI
IGF-I, ng/mL	0.02	-0.02	-0.01
Cortisol, ng/mL	0.22*	0.23*	0.29*
RBC, x 10^6/μL	0.18*	0.08	0.15*
WBC, x 10^3/μL	-0.03	0.03	0.01
Hemoglobin, g/dL	0.22*	0.09	0.18*
Total protein, g/dL	0.01	-0.01	0.00
Blood urea nitrogen, mg/dL	0.02	-0.06	-0.03
BHB, μmol/L	-0.15*	-0.22*	-0.25*
Glucose, mg/dL	0.27*	0.47*	0.49*
Creatinine, mg/dL	0.12*	0.11*	0.15*
GGT, U/L	0.00	-0.06	-0.05
AST, U/L	0.09	0.03	0.08

[1]IGF-I = insulin-like growth factor-I; RBC = red blood cell; WBC = white blood cell; BHB = beta-hydroxybutyrate; GGT = gamma-glutamyltransferase; AST = aspartate transaminase.

[2]REV = relative exit velocity; RCS = relative chute score; TI = temperament index.

*Correlations are different from zero at $P < 0.05$.

more excited, BHB concentrations decreased. This is contrary to what is expected. Heifers with a higher temperament rating would be more agitated and therefore under stress which causes the body to initiate lipolysis and protein degradation which would result in higher levels of BHB. Initial REV, initial RCS and initial TI were positively correlated with glucose (r = 0.27, 0.47, 0.49) and creatinine (r = 0.12, 0.11, 0.15) and of these 3 traits, initial TI had the highest correlation coefficients. King et al. (2006) and Schuehle Pfeiffer (2009) reported use of a TI as a means to rank animals based on multiple temperament traits and have reported significant correlations, however, both used the average of EV and pen score to calculate TI whereas in this study, we used EV and CS. Initial REV and TI were positively correlated with RBC counts (r = 0.18, 0.15) and HB levels (r = 0.22, 0.18) such that calmer heifers (low TI) had lower RBC and HB levels.

Appendix A reports weaning, 0 d and 70 d exit velocity correlations with serum metabolites. Wean REV, Initial REV, and Final REV were all positively correlated with cortisol concentrations (r = 0.20, 0.22, 0.29), and glucose levels (r = 0.18, 0.27, 0.36).

This indicates that temperamental heifers have higher levels of cortisol which induces gluconeogenesis and therefore increase glucose levels in the blood. Petherick et al. (2009) found similar results with multiple EV being positively correlated with glucose and cortisol concentrations. Similarly, King et al. (2006) recorded EV at three different time points and found pre-shipping EV, arrival EV, and midpoint EV all to be correlated with final cortisol concentrations (r = 0.30, 0.28, 0.41, respectively). That study also reported correlations for EV taken at 3 time points with pre-shipping CORT,

arrival CORT, and final CORT and found significant correlations with all except for Midpoint EV with pre-shipping CORT. The significant correlations at the 3 time points indicates that even if EV decreases over time, as animals become accustomed to production practices, EV still provides an reliable objective representation of animal stress responsiveness on temperament. Initial REV and final REV were positively correlated with RBC counts (r = 0.17, 0.13) and HB levels (r = 0.22, 0.17) and negatively correlated with BHB (r = -0.15, -0.17).

Temperament Index Groups

Differences in performance and feed efficiency traits between heifers with divergent temperament indexes are reported in Table 3.10. Final BW and MBW were statistically different amongst TI groups in that low temperament index heifers (calm) had 3% higher final BW and MBW. Similarly, low TI heifers had an 8% increase in ADG, a 4% increase in DMI and a 4% decrease in FCR compared to high TI heifers. These results are expected base on previous temperament studies.

When we look at the difference in serum metabolites amongst low and high temperament index groups, Table 3.11, there are significant differences in IGF-I, WBC, TP, BUN, or liver enzymes (AST and GGT). Temperament indexes were intended to be a predictor of stress responsiveness therefore, significant differences in cortisol concentrations between TI groups was expected. Low TI heifers had 28 % lower levels of cortisol compared to high TI heifers (3.94 vs. 5.43 ng/ml). Red blood cell counts and HB also differed amongst TI groups such that low TI heifers had 5% lower RBC counts and 4% lower HB concentrations. Beta-hydroxybutyrate concentrations were higher in

Table 3.10 Trial adjusted performance and feed efficiency traits for Brangus heifers with divergent temperament phenotypes[1]

Trait[2]	Low-TI	High-TI	SE	P-Value
Initial BW, kg	273.7	268.6	3.3	0.13
Final BW, kg	344.7	336.0	3.8	0.02
MBW, kg	73.75	71.46	0.62	0.001
ADG, kg/d	1.04	0.96	0.02	0.001
DMI, kg/d	9.65	9.22	0.13	0.001
FCR, DMI/gain	9.44	9.75	0.16	0.05
RFIp, kg/d	0.03	0.02	0.09	0.93
RFIc, kg/d	0.01	0.04	0.09	0.74

[1]Heifers with low and high TI were < 0.50 SD and > 0.50 SD from the mean TI of 0.00 ± 0.25, respectively.

[2]FCR = feed conversion ratio; RFIp = residual feed intake from base model; RFIc = residual feed intake from carcass-adjusted model.

Table 3.11 Trial adjusted serum metabolites traits for Brangus heifers with divergent temperament phenotypes[1]

Trait[2]	Low-TI	High-TI	SE	P-Value
IGF-I, ng/mL	130.9	127.2	4.3	0.39
Cortisol, ng/mL	3.94	5.43	0.22	0.001
RBC, x 10^6/μL	9.83	10.32	0.13	0.001
WBC, x 10^3/μL	10.01	9.86	0.31	0.63
Hemoglobin, g/dL	12.58	13.17	0.13	0.001
Total protein, g/dL	6.32	6.37	0.14	0.71
Blood urea nitrogen, mg/dL	11.06	10.92	0.23	0.55
BHB, μmol/L	269.6	228.5	8.7	0.001
Glucose, mg/dL	86.1	106.6	2.3	0.001
Creatinine, mg/dL	1.05	1.11	0.02	0.004
GGT, U/L	14.65	14.55	0.72	0.88
AST, U/L	20.61	23.06	1.73	0.16

[1]Heifers with low and high TI were < 0.50 SD and > 0.50 SD from the mean TI of 0.00 ± 0.25, respectively.

[2] IGF-I = insulin-like growth factor-I; RBC = red blood cell; WBC = white blood cell; BHB = beta-hydroxybutyrate; GGT = gamma-glutamyltransferase; AST = aspartate transaminase.

the low TI group (269.6 vs. 228.5 μmol/l) and had lower levels of glucose and creatinine concentrations (86.1 vs. 106.6 mg/dL and 1.05 vs. 1.11mg/dL) compared to their high TI counterparts.

Implications

Based on previous research, approximately 60 % of the variation in DMI is explained by BW and ADG. Residual feed intake is an efficiency measure that is independent of MBW and ADG making it useful in the selection of efficient cattle without affecting body size and maintenance requirements. Physiological biomarkers or indicator traits of economically relevant traits (ADG, intake) may be predictive of RFI and would facilitate early identification and more accurate selection of calves with superior genetic merit for RFI. However, research evaluating the relationships between RFI with temperament and serum metabolites has generated inconsistent results. Based on this research, serum metabolites have limited utility for use as indicator traits for RFI in growing heifers.

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APPENDIX A

Table A.1 Phenotypic correlations among temperament traits with trail adjusted performance and feed efficiency traits in Brangus heifers

Trait[1]	Wean REV[2]	Initial REV	Final REV
Initial BW, kg	-0.14*	-0.11*	-0.11*
Final BW, kg	-0.11*	-0.13*	-0.15*
MBW, kg	-0.20*	-0.24*	-0.22*
ADG, kg/d	0.01	-0.16*	-0.17*
DMI, kg/d	-0.12*	-0.21*	-0.18*
FCR, DMI/gain	-0.12*	0.01	0.04
RFIp, kg/d	-0.05	-0.05	-0.03
RFIc, kg/d	-0.01	-0.03	-0.03

[1]FCR = feed conversion ratio; RFIp = residual feed intake from base model; RFIc = residual feed intake from carcass-adjusted model.

[2]REV = relative exit velocity.

*Correlations are different from zero at $P < 0.05$.

Table A.2 Phenotypic correlations among temperament traits with trial adjusted serum metabolites in Brangus heifers

Trait[1]	Wean REV[2]	Initial REV	Final REV
IGF-I, ng/mL	-0.13*	0.02	-0.06
Cortisol, ng/mL	0.20*	0.23*	0.29*
RBC, x 10^6/μL	0.06	0.17*	0.13*
WBC, x 10^3/μL	-0.07	-0.03	-0.09
Hemoglobin, g/dL	0.05	0.22*	0.17*
Total protein, g/dL	0.03	0.01	0.04
Blood urea nitrogen, mg/dL	-0.06	0.02	0.00
BHB, μmol/L	-0.09	-0.15*	-0.17*
Glucose, mg/dL	0.18*	0.27*	0.36*
Creatinine, mg/dL	0.03	0.12*	0.10
GGT, U/L	-0.04	0.00	0.06
AST, U/L	0.07	0.10	0.07

[1]IGF-I = insulin-like growth factor-I; RBC = red blood cell; WBC = white blood cell; BHB = beta-hydroxybutyrate; GGT = gamma-glutamyltransferase; AST = aspartate transaminase.

[2]REV = relative exit velocity.

*Correlations are different from zero at $P < 0.05$.

APPENDIX B

Table B.1 Percentage of variance (R^2) in feed intake explained by the inclusion of serum parameters

	Model Number[1]		
Regression[2]	$F2+e_1$	$F1+R+e_2$	$(F1+R)-R+e_3$
Base model (BM; ADG and MBW)	0.472	0.538	0.531
BM + Blood urea nitrogen	0.486	0.541	0.535
BM + Creatinine	0.479	0.544	0.536
BM + WBC	0.490	0.549	0.542
BM + Total protein	0.477	0.545	0.538
BM + Glucose	0.474	0.538	0.531
BM + Hemoglobin	0.481	0.544	0.539
BM + BHB	0.478	0.546	0.539

[1] F1= fixed effects of indicated variables; F2 = fixed effects of indicated variables + fixed effect of test; R = random effects of test and test by independent variable interactions; e_1 = random test and uncontrolled error; e_2 = random uncontrolled error and error associated with fixed interactions of test and independent variables; e_3 = random uncontrolled error.

VITA

Name:	Robynne Ryan Gomez
Address:	2471 TAMU, College Station, TX 77843
Email Address:	robynne99@gmail.com
Education:	B.S., Agribusiness, Sam Houston State University, 2004 M.S., Animal Science, Texas A&M University, 2010